THE DEVELOPMENT OF THE ASCIDIAN EGG CENTRIFUGED BEFORE FERTILIZATION

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The development of centrifuged eggs of ascidians has been studied by Duesberg (1926) and by Conklin (1931). In *Styela* and *Ciona* Conklin found that when the eggs were centrifuged after fertilization, three substances, namely the mitochondria, the hyaloplasm, and the yolk may be displaced from their normal positions into three zones. If these substances were so held until the beginning of cleavage, they were distributed abnormally to the blastomeres. The tissues or organs of the embryos derived from these eggs were also dislocated.

The unfertilized eggs of *Styela* and *Ciona* also were centrifuged by Conklin. He noted that under strong centrifuging the spermatozoön frequently did not enter the egg at all. No description of the development of these eggs was given.

Recently Dalcq (1932, 1935, 1938) has fragmented unfertilized eggs of *Ascidiella scabra* into two parts and then fertilized them. He found that meridional halves of egg fragments may give rise either to apparently normal and symmetrical larvae, or to lateral half larvae; while larvae obtained from latitudinal halves may be deficient in one or more kinds of tissues according to the level of the cut. These experiments indicate that the unfertilized egg already possesses germinal localizations; but at the same time it has a great capacity for regulation, as has been demonstrated by Tung (1934) for the fertilized egg.

To gain further light on the organization of the unfertilized egg of the ascidian and to learn whether, with sufficient force, the organ-forming substances might be dislocated, we have made a study of the development of *Ciona* eggs strongly centrifuged before fertilization. The experiments on which this investigation is based were performed in the summer and autumn of 1936.

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Material and Methods

The observations recorded here were all made on eggs of *Ciona intestinalis* obtained in the vicinity of Tsingtao. During the months from June to October the gonoducts of *Ciona* are usually full of ripe eggs and spermatozoa. The eggs were obtained free from sperm by removing the integuments and carefully opening the oviduct which is near the surface. The eggs were then removed from the oviduct with a pipette and transferred to a glass dish containing fresh sea water. A part of the eggs was used for centrifuging, leaving the remaining eggs in the dish as a control.

The eggs to be centrifuged were placed in glass tubes with 10 cc. sea water and rotated for ten minutes to one hour and fifty minutes at a speed ranging from 2000 r.p.m. to 3800 r.p.m. These speeds represented centrifugal forces of about $716 \times$ gravity and $2585 \times$ gravity. In order to prevent eggs from rotating during centrifugation, capillary tubes were used as by Conklin. After centrifuging, the eggs were immediately removed from the capillary tubes and fertilized by sperm of another animal.

Individual eggs showing an abnormal distribution of oöplasmic substances to the first two or four blastomeres were picked out, sketched, and placed in separate dishes of fresh sea water in order to study in detail the location of various tissues in later development.

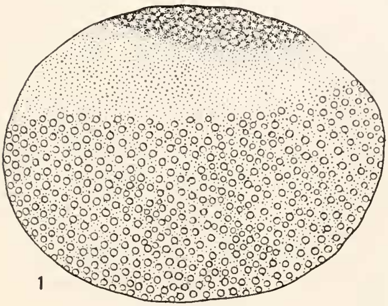
The larger part of the eggs and embryos was fixed in Bouin's fluid and double-embedded in agar-paraffin (Chatton, 1927). Sections were cut at $7\,\mu$ and stained with iron-haematoxylin, eosin, and light green. Some of the material was fixed in Flemming's solution and mounted in toto in order to determine the location of mitochondria.

Results

Stratification of the Egg

The degree of the stratification of oöplasmic substances of the unfertilized egg varies with the rate and duration of centrifugation. In strongly centrifuged eggs three zones can be distinguished. These are: (1) an alveolar or light zone at the centripetal pole; (2) a middle clear zone containing the nuclear elements; and (3) a lower, heavy zone of yolk. In eggs fixed in Flemming's solution and mounted in toto, the light zone consists exclusively of large black, densely packed granules. On the basis of the staining reaction, it corresponds obviously to the "mitochondria" zone of Duesberg. Sections of the eggs show that this zone is composed of an alveolar substance in which are embedded a large number of blue-black granules; these are taken to be mitochondria (Fig. 1).

Under prolonged centrifuging, a fourth zone appears at the centrifugal pole. Figure 2 shows an egg in which the four zones are



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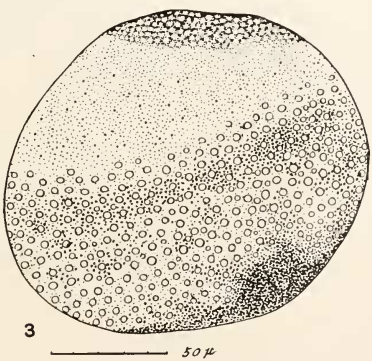

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Fig. 1. Section of an unfertilized egg, centrifuged for one hour at 2193 times gravity, and fixed ten minutes later, showing a stratification of the egg contents into three zones.

Fig. 2. Section of an unfertilized egg centrifuged for one hour at 2193 times gravity and fixed ten minutes later, showing the fourth zone at the centrifugal pole.

Fig. 3. Section of an unfertilized egg, centrifuged for one hour at 2193 times gravity and fixed fifteen minutes later. The stratification of the centrifugal zone is not complete.

clearly separated. The centrifugal zone contains blue-black granules, while in the centripetal zone the number of the same kind of granules is greatly reduced. Figure 3 shows an egg with incomplete stratification of the fourth zone. In this case a part of the granules remains scattered throughout the clear and yolk zones and especially in the latter. A comparison of the eggs illustrated in Figs. 1, 2, and 3 leaves no doubt that the granules of the centrifugal zone are separated out from the alveolar substance of the centripetal zone in which they were formerly embedded.

Conklin described a fourth zone between the hyaline and yolk zones and concluded that ". . . It (the substance of the zone), rather than the mitochondria, is the myoplasm or formative substance for the future muscles." Our conclusions are that it is the alveolar substance of the centripetal zone that is the formative substance of the muscles; the alveolar substance is similar to the cytoplasm of muscle cells of the young embryo both in structure and in staining reaction, whereas the granules

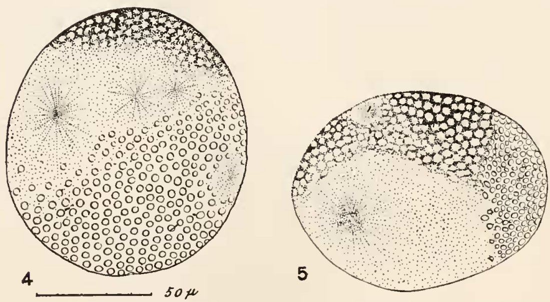


Fig. 4. Section of a polyspermic egg centrifuged for one hour at 2193 times gravity and fixed fourteen minutes after fertilization. Note sperm asters in clear and yolk zones.

Fig. 5. Section of a polyspermic egg centrifuged for one hour at 2193 times gravity and fixed fourteen minutes after fertilization. Note sperm asters in clear and alveolar zones.

of the centrifugal zone are the mitochondria since in normal fertilized eggs similar granules are found in the lower hemisphere at which the sperm enters. In *Physa heterostropha,* Clement (1938) has recently shown that the mitochondria which stratify between the clear and yellow zones and are therefore heavier than the clear protoplasm are the last to be segregated under centrifugal forces. In these respects our observations on *Ciona* agree with those of Clement on *Physa.*

Immediately after centrifuging, the eggs were cross-fertilized with fresh sperm. As a check, in each experiment, the control eggs left unfertilized in the original dish were examined. As has recently been reported by Morgan (1938), cleavages were rare, showing that self-fertilization occurs rarely in *Ciona.* Conklin found that in eggs which had been strongly centrifuged before fertilization, the spermatozoön frequently did not enter at all. He attributed the failure of fertilization to the compactness of the yolk at the vegetative pole which blocked the entrance of the spermatozoön. In our experiments, though we have no detailed record, the percentage of fertilization in centrifuged eggs is generally not very low in comparison with normally fertilized eggs.

In sections it appears that the spermatozoön may penetrate the egg in the clear zone or between this zone and the yolk; for the most part it enters the yolk zone (Fig. 8). This indicates that the compactness of the yolk is not a factor in blocking the entrance of the spermatozoön. In polyspermic eggs the sperm asters are found in almost any part of the egg, even in the alveolar substance of the centripetal zone. Figures 4 and 5 show two such eggs that had been centrifuged for one hour at 2193 $\times$ gr. and were fixed fourteen minutes after fertilization. The entrance points of the sperm are suggested by the positions of the asters.

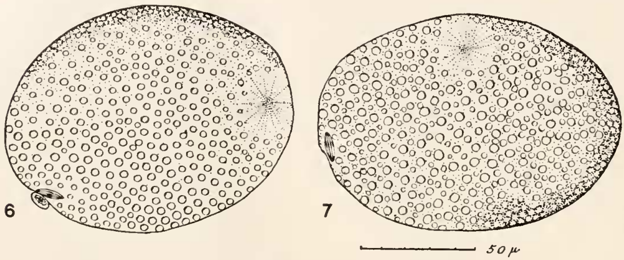
The first maturation spindle of the strongly centrifuged eggs always lies in the clear zone. After fertilization it moves to the periphery of the zone where the polar bodies are given off. In some polyspermic eggs, when a sperm-nucleus has already migrated to the center of the egg, the first maturation spindle still rests in the middle of the clear zone. In such cases probably no polar bodies will be given off; they will be retained in the egg.

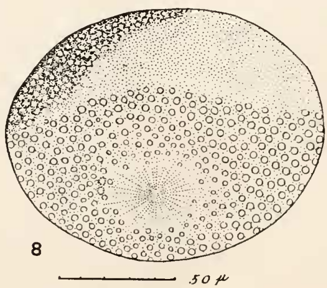
The entrance point of the spermatozoön may be found at any plane with respect to the position of polar bodies. This is evident in Figs. 6 and 7, which represent two eggs centrifuged for ten minutes and fixed one hour after fertilization.

In the normal egg a protoplasmic movement usually takes place immediately after the entrance of the sperm. Such movements probably also occur in the weakly centrifuged eggs with incomplete stratification of oöplasmic substances, since after fertilization the different substances of such eggs return to their normal positions. In strongly centrifuged eggs the movement of the oöplasm is less marked. This is well demonstrated in Fig. 8. This egg was centrifuged in a capillary tube for one hour and twenty-four minutes at 2193 $\times$ gr., and fixed forty minutes after fertilization. The three principal zones are still quite clear. The sperm aster is found in the yolk zone surrounded by a clear substance and no mitochondria. It appears that the free movement of the substances is impeded by the stratification.

Cleavage

As a check, in every experiment some of the control eggs were fertilized at the same time as the centrifuged eggs. The first cleavage of the eggs of both sets occurred at the same time, regardless of the rate and duration of centrifuging. It took place usually in about one hour and a half after insemination, much later than the records of other authors. Such delayed cleavage may be due to the low temperature of the sea water at Tsingtao.

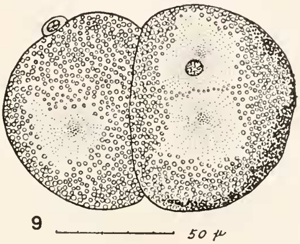




FIGS. 6 and 7. Sections of eggs centrifuged for ten minutes at 2193 times gravity and fixed one hour after fertilization, showing the positions of the sperm aster, the polar body (Fig. 6) or second maturation spindle and the mitochondria crescent. There are no constant relations between them.

FIG. 8. Section of an egg centrifuged in capillary tube for one hour and twenty-four minutes and fixed forty minutes after fertilization. The sperm aster is in the yolk zone; no mitochondria surround it.

The pattern of the cleavage planes of the centrifuged eggs differed in no essential respect from those of normal eggs. The first two cleavage planes were perpendicular to each other, while the third plane was at right angles to both of the first two, resulting in the formation of four micromeres and four macromeres. However, the first two cleavage planes did not always pass through the point of attachment of the polar bodies. After prolonged centrifuging, there was a significant percentage of eggs whose polar bodies were not at the first cleavage furrow. The deviation between them may be of any angle up to 90° (Fig. 9). Since after centrifuging there was no definite landmark to indicate the original position of the animal pole and since the orientation of the eggs during the process of centrifuging could not be determined accurately, it was impossible to ascertain whether the polar bodies were



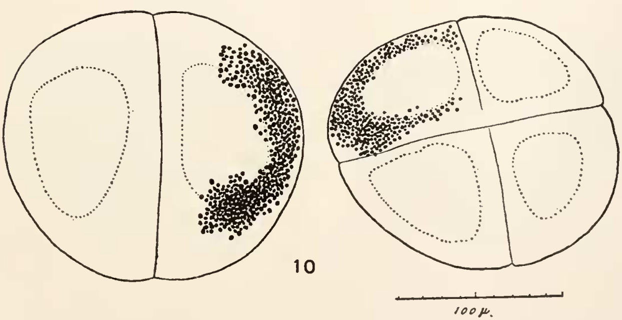
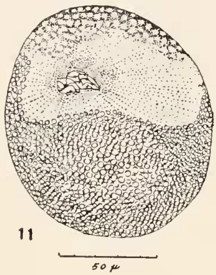


Fig. 9. Section of an egg in 2-cell stage. The polar body is not located at the cleavage furrow.

Fig. 10. Two entire eggs in 2- and 4-cell stages, showing mitochondria in one blastomere.

produced at other than their normal position, or whether the first cleavage plane passed through the original animal pole.

In most eggs the first cleavage plane appears to coincide with the axis of centrifugation. As the stratified substances are rarely equally distributed around the axis, it divided them into more or less unequal halves. However, there is a significant percentage of eggs in which the first cleavage appeared in any plane and at any angle with respect to the stratification of substances. It may be oblique to the axis of centrifugation or perpendicular to it. In the latter case one blastomere contains only the yolk spherules and the other the clear and alveolar substances. Figure 10 shows two such eggs in the 2- and 4-cell stages respectively. They were fixed in Flemming's solution and mounted in toto. The mitochondria or alveolar substance is confined to one blastomere. Similar conditions are also shown in Fig. 9.



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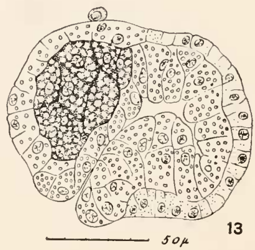

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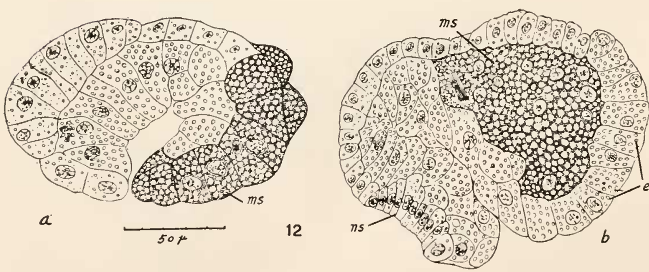

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FIG. 11. Section of an egg centrifuged for one hour at 2193 times gravity. The three oöplasmic zones remain unchanged after fertilization.

FIG. 12. Section of two gastrulae, showing the abnormal positions of muscle (*a*) and endoderm (*b*) cells. *ms.*, muscle cell; *end.*, endoderm; *ns.*, neural cell.

FIG. 13. Section of a gastrula derived from egg centrifuged fifteen minutes at 2193 times gravity. The polar body is situated posterior to the middle of the ventral surface in its typical position.

It not infrequently happens that the first cleavage is quantitatively unequal; this leads to a formation of a larger macromere and a smaller micromere. Sometimes the second cleavage plane does not intersect the first at right angles, so that the four resulting blastomeres do not lie in the same plane. The cleavage pattern in such eggs as well as in those described above seems to be determined by the position of the cleavage spindle. In cases where the three principal zones remain unchanged after fertilization, the first cleavage spindle always lies horizontally in the clear zone. In such eggs the cleavage plane coincides with the axis of centrifuging. If a slight change of the stratification takes place before the division, the position of the mitotic spindle is also changed. The subsequent cleavage will then divide the egg in any plane with respect to the axis of the centrifuging. Figure 11 shows a section of an egg in which the oöplasmic substances were stratified into three zones. The two pronuclei had come together in the center of the clear zone. The first cleavage of this egg may be expected to approximate the axis of stratification and will distribute the oöplasmic substances equally between the two blastomeres.

In many eggs the division of the cell body is suppressed while the division of centers and chromosomes continues. In such eggs are found numerous nuclei and centers, confined to the alveolar and clear zones. Such anomaly of cleavage is evidently not due to any direct effect upon the mitotic figure, for the fertilization of the eggs took place after centrifuging.

Later Development of Centrifuged Eggs

The later development of centrifuged eggs is particularly interesting since the dislocated tissues or organs can be identified in stained sections. Individual eggs showing abnormal distribution of oöplasmic substances to the first two, four, or eight blastomeres were sketched and isolated. The development of these eggs was studied. In general, it may be said that the stronger and the longer the centrifuging, the more abnormal was the subsequent development.

The gastrulation of strongly centrifuged eggs was rarely typical. Figure 12 shows two abnormal cases in which a part of the mesoderm and entoderm cells had not been invaginated. In normal development the polar bodies are situated on the anterio-ventral part of the older gastrula. In Fig. 13 a polar body is found posterio-ventrally on the gastrula, indicating that gastrulation took place independent of the position of the polar bodies.

Following prolonged centrifugation, a large number of eggs started their development but most of them never reached a stage at which they could be recognized as larvae. The embryos were so abnormal in form that it was impossible to identify their parts and organs except by a histological study. In these embryos, tissues and organs which will be described separately in the following paragraphs were usually displaced. Embryos are found composed of three types of cells, namely: (1) muscle and mesenchyme cells; (2) ectoderm and neural cells; and (3) notochordal and endoderm cells. These types correspond to the products of the three zones (viz., alveolar substance, clear cytoplasm, and yolk) which had been stratified by the centrifugal force.

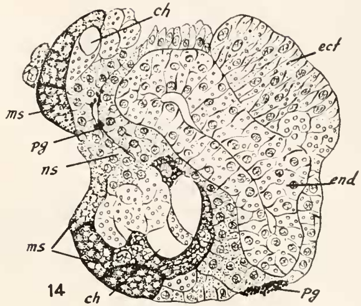
Muscle Cells and Mitochondria.—In typical embryos and larvae, the large muscle cells are arranged in the tail in three rows on each side of the notochord. Owing to their large nuclei and specific staining reaction, they are easily distinguished from other types of cells. In the abnormal larvae derived from centrifuged eggs, isolated or aggregated cells of this type may be found in the interoir or at the surface (Fig. 14). They are rarely arranged regularly even when they are found alongside of the notochord. In some cases these cells lie in the midst of the endoderm or just under the neural tissue. Figure 15 shows a section of a larva in which a part of the gut wall is formed of cells other than typical endoderm. Though one cannot be certain as to whether these are really mesoderm cells or not, the structure of the cytoplasm and the size of the nucleus are similar to those of the latter.

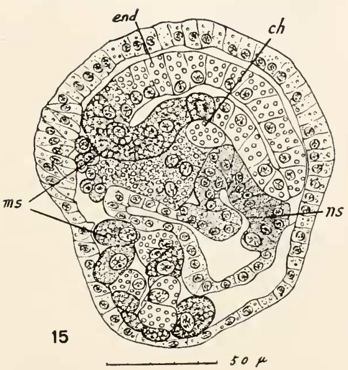
The mitochondria which are normally embedded in the cytoplasm of the muscle cells may have been driven into the regions subsequently forming ectoderm (Figs. 16 and 19*a*) or neural tissue (Fig. 15). The myofibrillae are often found in the muscle cells, but are never found in those tissues into which the mitochondria have been driven. Our observations, therefore, confirm the view of Conklin, that in *Ciona* the mitochondria do not give rise directly to myofibrillae.

Notochordal Cells.—Owing to the abundance of yolk spherules, the notochordal cells of the young embryo are very similar to those of the endoderm. In larvae, on the other hand, they are characterized by the possession of large vacuoles and can be thus recognized wherever they occur. In most abnormal larvae derived from strongly centrifuged eggs, notochordal cells did not arrange themselves to form a rod, but instead were displaced to various positions. They may be grouped together or scattered. In some cases, they are found at the surface (Fig. 17) of the larvae or just under the ectoderm (Fig. 16). In other cases they are in the midst of endoderm or surrounded by muscle cells. In such embryos no tail is formed.

The neural tissue which will be described in the next paragraph was not always associated with the notochordal cells; nor do the latter exert any influence on the differentiation of the ectoderm cells with which they are in contact. We agree, therefore, with Berrill and Conklin who have concluded that the notochord of the ascidian does not act as an organizer in the sense of Spemann.

Neural Cells and the Sensory Pigment.—The neural cells may occur in any portion of these abnormal larvae. Sometimes they formed a





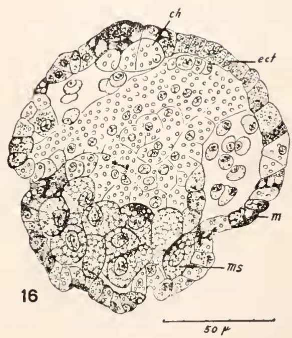
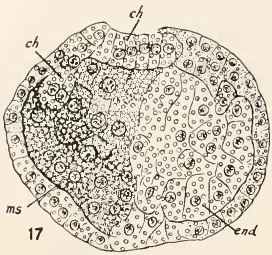


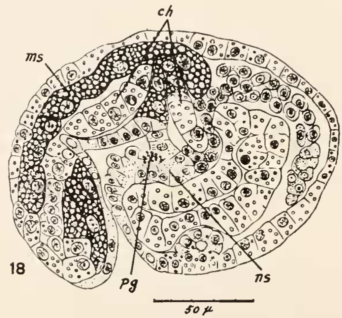
FIG. 14. Section of an abnormal larva derived from an egg centrifuged for thirty minutes at 2193 times gravity and fixed twenty-eight hours after fertilization. *ms.*, muscle cell; *ch.*, notochord; *pg.*, pigment spot; *ns.*, neural cell; *ect.*, ectoderm; *end.*, endoderm.

FIG. 15. Section of an abnormal larva derived from an egg centrifuged for one hour and thirty minutes at 2193 times gravity and fixed twenty-four hours after fertilization. A part of the gut wall contains alveolar substance. *ms.*, muscle cell; *ns.*, neural cell; *end.*, endoderm; *ch.*, notochord.

FIG. 16. Section of a larva from an egg centrifuged for one hour and fifty minutes, showing the mitochondria in the ectoderm and neural cells. *m.*, mitochondria; *ms.*, muscle cell; *ch.*, notochord.

plate on the surface; at other times they were grouped forming a mass of neural tissue. Usually, however, they lined a large irregular cavity extending into the interior of the larva. In larvae having well-developed tails, such a neural cavity is always found at the junction of the trunk and the tail. The latter often turned dorsally, giving the larvae a curvature in an atypical direction. Figure 18 shows a larva of this kind. The neural cavity with two spots of sensory pigment is surrounded by the curved tail on one side. Conklin has suggested that the elongation of the neural plate and tube depends upon the normal elongation of the notochord. Our observations, however, do not lead to this conclusion.





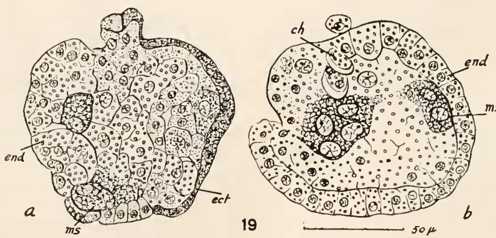


FIG. 17. Section of a larva from an egg centrifuged in capillary tube for forty minutes at 2193 times gravity. *ch.*, notochord; *ms.*, muscle cell; *end.*, endoderm.

FIG. 18. Sagittal section of a larva derived from an egg centrifuged one hour and thirty minutes at 2193 times gravity, showing the inverse body curvature. *ch.*, notochord; *ns.*, neural tissue; *pg.*, pigment spot; *ms.*, muscle cell.

FIG. 19. *a.* Section of a larva from an egg centrifuged in a capillary tube for fifty minutes at 2585 times gravity. The left yolk-filled cells are endoderm and the right mitochondria-filled cells are ectoderm.

b. Section of a larva from an egg centrifuged for fifteen minutes at 2193 times gravity. The yolk-filled cells form a superficial epithelium. *ch.*, notochord; *ect.*, ectoderm; *end.*, endoderm; *ms.*, muscle cell.

The sense organs are also structures of interest. In typical larvae there are two sense organs, the eye and the otocyst, with their pigments situated on the wall of the brain vesicle. Typical organs are rarely formed in abnormal larvae; instead, pigment spots are found in unexpected places. The number of spots varies from none to four. Their locations may be near together or widely separated (Fig. 14). In some cases they project from the surface of the larva and in others they are embedded in the neural cells. However, they are always associated with neural tissue and notochordal cells.

Endoderm and Ectoderm Cells.—The histological characteristic of endoderm is its large yolk-filled cells. In these abnormal embryos, however, it is not easily identified, for the yolk spherules are also found in other cells of the embryo. In such a case as that shown in Fig. 19*A* there is no doubt but that the superficial cells of the left side are endodermal and those of the right side are ectodermal. In the case shown in Fig. 19*B*, on the other hand, one cannot be certain as to whether the yolk-filled surface epithelium labeled " end " is true endoderm or is ectoderm. Tung (1934), in experiments on the combination of blastomeres in *Ascidiella scabra,* has shown that the development of ectoderm and endoderm is not strictly mosaic. When the endodermal cells lie at the surface of the embryo, they may form a regular superficial epithelium. Similarly, if ectodermal cells come to lie in the interior, they may transform into large irregular endoderm-like cells. Such a regulative capacity of ectoderm and endoderm seems to exist also in the tissues of *Ciona* embryos.

In these larvae having displaced organs, the endodermal cells may be found in many abnormal locations. The fact that they are not always associated with neural tissue and pigmented sensory spots indicates that they do not serve as organizers.

Papillae.—The normal embryo possesses three papillae; two are paired and are situated dorso-laterally on each side; one is median and ventral. Papillae are also observed in some of the abnormal larvae. Their number varies from none to three. It is of interest to note that in spite of the dislocation of other organs, the development of papillae is always in relation to the endoderm. Without exception in all 49 cases observed, the papillae were formed in ectoderm underlain by endoderm. Tung (1934), on the basis of experiments on *Ascidiella scabra* in which the four animal blastomeres were rotated through 180° over the four vegetal blastomeres, has suggested that papillae are evoked in the ectoderm by underlying endoderm. The present observation furnishes further evidence in support of that hypothesis.

From the above descriptions it is obvious that the different tissues and organs of the many larvae derived from eggs centrifuged before fertilization, are out of their normal positions. Such placement of tissues is undoubtedly the result of the placement of the oöplasmic substances from which the tissues are derived. In some cases the quantity of certain tissues seems to be reduced. We have not, however, made a count of the cellular components of these tissues.

Discussion

Conklin (1905 *a* and *b*), in his classical studies on the development of ascidians, described four different kinds of oöplasmic substances in the fertilized egg, namely the ectoplasm, endoplasm, mesoplasm, and chorda-neuroplasm. From these are derived the ectoderm, endoderm, mesoderm, and the notochordal and neural tissue respectively. According to Conklin (1905*c*), the development of these tissues or organs is strictly mosaic. When the eggs were centrifuged after fertilization, tissues of the larvae which developed were displaced from their normal positions (1931). Such displacements of tissues were attributed to corresponding displacements of oöplasmic substance by means of centrifugal force.

As has been shown in the present experiments, the stratification of oöplasmic substances of eggs centrifuged prior to fertilization and the displacement of the tissues or organs of the larvae derived from these eggs are in general similar to those obtained from eggs centrifuged after fertilization. It is reasonable, therefore, to conclude that the different kinds of oöplasmic substance described by Conklin in fertilized eggs have already existed in the egg before fertilization. This conclusion agrees with that of Dalcq (1932, 1935, 1938), who likewise concluded that organ-forming substances are already differentiated in the unfertilized eggs. Conklin has suggested that the unfertilized egg of *Cynthia* possesses a bilateral symmetry. If so, it is undoubtedly due to the bilateral arrangement of these oöplasmic substances.

In recent years the idea that the ascidian egg is strictly mosaic has been called in question, though it is still insisted upon by Berrill (1932) and by Cohen and Berrill (1936). Schmidt (1931) observed three papillae in larvae derived from one-half blastomeres of *Ciona* and *Phylusia* eggs. Reverberi (1931) has obtained normal larvae from fragments of fertilized *Ciona* eggs. Tung (1934) has found in *Ascidiella scabra* that the endoderm and ectoderm are relatively equipotential and that the development of papillae and sensory pigments is always associated with endoderm and notochord respectively. Recently, Von Ubisch (1938) has described a normal embryo of *Ascidiella aspersa* produced by fused eggs. All these investigations show that after fertilization the ascidian egg has a considerable capacity for regulation.

Dalcq has reported a certain degree of regulation in the unfertilized egg of *Ascidiella scabra*. A fragment of the egg may give rise to an embryo very similar to the control. The number of cells of muscles or notochord of a pair of embryos derived from two fragments of one egg may be double the total number of a normal embryo. Moreover, in the experiments reported here, we have demonstrated that the ectoderm and endoderm constitute relatively equipotential systems and that the papillae and sensory pigment do not appear to be self-differentiating organs. These facts plainly show that the eggs of *Ascidiella* and *Ciona* are not strictly mosaic prior to fertilization.

The recent work of Rose (1939) shows that the anterior vegetal region of the *Styela* egg is the cerebral inductor. This region contains materials essential to the differentiation of endoderm, notochord and a part of the neural tissue. In the larvae with displaced tissues or organs we have not found any typical relations between the cerebral vesicle and either notochord or endoderm. In short, there is no evidence in the present experiments to indicate that either notochord or endoderm acts as a cerebral inductor. The development of the adhesive papillae has been discussed by Cohen and Berrill (1936). They have found three papillae in a lateral half larva of *Ascidiella aspersa* and interpret the origin of such supernumerary papillae as a result of the mosaic pattern. If this interpretation is correct, it might be expected that in larvae with displaced organs, papillae would be found in a variety of abnormal locations. On the contrary, papillae in abnormal larvae are always associated with the endoderm. This fact furnishes further evidence in favor of the suggestion of Tung that papillae appear to be evoked in the ectoderm by the underlying endoderm.

From the foregoing discussion we come to the conclusion that the organization of the unfertilized egg of the ascidian is similar to that of the fertilized egg. In both there exist different kinds of oöplasmic substances from which different tissues develop. These substances, however, are not strictly mosaic; they still possess a certain capacity for regulation. Some organs, such as papillae and sensory cells, seem incapable of self-differentiation. Their development might be dependent upon extrinsic factors.

Summary and Conclusions

1. The unfertilized eggs of *Ciona intestinalis* after centrifuging can be cross-fertilized. The majority undergo cleavage but rarely develop normally.

2. The first cleavage furrow may lie in any plane relative to the position of polar bodies or the axis of centrifuging.

3. In larvae derived from strongly centrifuged eggs, tissues and organs were often displaced from their normal positions.

4. Endoderm and ectoderm appear to be relatively equipotential.

5. There is no evidence that the differentiation of the neural tissue is dependent upon other tissues with which it is in contact.

6. Mitochondria may be displaced from muscle cells and appear in

the neural or ectodermal cells, where they are not transformed into myofibrillae.

7. The number of papillae developed by the abnormal larvae varied from none to 3. They are always adjacent to the endoderm which it is suggested may evoke their formation in the ectoderm.

8. The number of sensory pigment spots in the abnormal larvae varies from none to 4. They are always associated with notochordal cells.

9. The organization of unfertilized eggs is found to be strikingly similar to that of fertilized eggs in respect to oöplasmic substances and the capacity of regulation.

10. The elongation of the notochord is not always accompanied by the elongation of the neural plate or tube.

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